

Solar and Temporal Effects on *Escherichia coli* Concentration at a Lake Michigan Swimming Beach†

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Studies on solar inactivation of *Escherichia coli* in freshwater and in situ have been limited. At 63rd St. Beach, Chicago, Ill., factors influencing the daily periodicity of culturable *E. coli*, particularly insolation, were examined. Water samples for *E. coli* analysis were collected twice daily between April and September 2000 three times a week along five transects in two depths of water. Hydrometeorological conditions were continuously logged: UV radiation, total insolation, wind speed and direction, wave height, and relative lake level. On 10 days, transects were sampled hourly from 0700 to 1500 h. The effect of sunlight on *E. coli* inactivation was evaluated with dark and transparent in situ mesocosms and ambient lake water. For the study, the number of *E. coli* samples collected (*n*) was 2,676. During sunny days, *E. coli* counts decreased exponentially with day length and exposure to insolation, but on cloudy days, *E. coli* inactivation was diminished; the *E. coli* decay rate was strongly influenced by initial concentration. In situ experiments confirmed that insolation primarily inactivated *E. coli*; UV radiation only marginally affected *E. coli* concentration. The relationship between insolation and *E. coli* density is complicated by relative lake level, wave height, and turbidity, all of which are often products of wind vector. Continuous importation and nighttime replenishment of *E. coli* were evident. These findings (i) suggest that solar inactivation is an important mechanism for natural reduction of indicator bacteria in large freshwater bodies and (ii) have implications for management strategies of nontidal waters and the use of *E. coli* as an indicator organism.

The survival of fecal indicator bacteria (*Escherichia coli*, enterococci) in ambient environments is strongly influenced by abiotic (e.g., salinity, sunlight, and temperature) and biotic (predation and competition) factors. The biological tolerance of *E. coli* to physicochemical factors has been especially well studied, albeit mostly in the laboratory. Of these factors, incoming solar radiation (insolation) is arguably the most potent in the inactivation or killing of *E. coli* and enterococci in water (6, 10, 17, 20, 21; R. S. Fujioka and E. B. Siwak, Am. Water Works Assoc., Water Qual. Technol. Conf., 1987).

Studies on solar inactivation of fecal indicator bacteria have been largely restricted to marine waters (8, 10, 20). Little research has focused on its effect on indicator bacteria in large freshwater bodies, such as the Great Lakes. Fresh and marine waters certainly share *E. coli* response characteristics to sunlight, but important differences justify separate study of each system. *E. coli* survives longer in freshwater (9), and the response curve to its natural decline—due to predation, competition by other microflora, and deposition in sediments (3, 12–15; M. W. Rhodes and H. I. Kator, Annu. Meet. Am. Soc. Microbiol. 1984, abstr. N 41, p. 185, St. Louis, Mo., 1984)—is compounded by salinity in marine water (5, 11, 22). Marine beaches are also subject to tidal changes, so elevated densities of *E. coli* in marine waters may be attributed to tidal cycles (4, 23), which also may compound diurnal changes associated with sunlight.

In nontidal waters, such as Lake Michigan, the diurnal solar effect is perhaps far more direct and easily discernible.

Ideally, it is desirable to conduct studies on the effect of insolation on indicator bacteria in situ, covering a range of diurnal, seasonal, and hydrometeorological factors that can potentially influence the interaction (e.g., sunny versus cloudy days; high versus low contaminant loadings, clear versus turbid waters, and tidal versus nontidal waters). Unfortunately, most studies on inactivation have been limited to the laboratory and usually consist of microcosm or mesocosm experiments lasting only a few days. Even though the results from such controlled experiments are important for determining the effect of an individual factor or a combination of factors on the inactivation processes, the relevance of these data to natural conditions has not been adequately tested. Moreover, the factors that affect this association under natural conditions are far more complex and dynamic, often characterized with additive, antagonistic, or synergistic effects (e.g., sunlight plus temperature or salinity) (22).

In fresh swimming waters, the processes and factors affecting solar inactivation of *E. coli* may directly impact management procedures designed to protect public health. The U.S. Environmental Protection Agency (EPA) has recommended a criterion limit for *E. coli* density in freshwater (235 CFU 100 ml⁻¹) in order to protect swimmers effectively from potential waterborne infection (24). The criteria do not take into consideration two factors—time of day and amount of insolation—that influence *E. coli* counts in the water. Monitoring samples are often taken early in the morning to accommodate analytical timetables, even though peak swimming activities are typically in the afternoon, so elucidation of the diurnal

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nature of indicator bacteria is valuable. From a management perspective, if developed protective criteria do not accurately reflect a reasonable indicator bacterial concentration at the time of exposure, the criteria merit reexamination or at minimum some readjustment to account for the time-dependent nature of the relationship.

The present study was undertaken at 63rd St. Beach, Chicago, Ill., located along southern Lake Michigan (N 41.78246, W 87.57301), to determine the diurnal fluctuations in *E. coli* abundance in beach water. Thus, the primary objective of this study was to characterize the factors, especially insolation, responsible for the daily periodicity of culturable *E. coli*. This study had four distinct components: (i) diurnal variations in *E. coli* counts were measured directly in the water on 10 days during the swimming season (May to September), (ii) time-dependent regression models were developed to determine *E. coli* decline patterns in response to light exposure, (iii) the daily relationship of morning and early afternoon insolation and the corresponding *E. coli* concentration were examined from late spring to early fall, and (iv) the above relationships were validated by using an in situ mesocosm study to measure diurnal changes with and without direct sunlight.

MATERIALS AND METHODS

Study area. The study area was located on the southwest shore of Lake Michigan at 63rd St. Beach, Chicago, Ill. The study area features are explained in significant detail elsewhere (26). In brief, the beach is approximately 12 km south of downtown Chicago, within the Jackson Park area. On the north end of the beach, a stone revetment extends along the shore, ending at Jackson Harbor and an associated breakwater. The southern end of the beach ends at Casino Pier, a doglegged breakwater that partially encloses the beach basin. The 63rd St. Beach is a popular beach along the lakefront, attracting local visitors during the recreation season (May to September).

Daily sampling. Grab water samples for *E. coli* enumeration were taken by dipping a sterile polyethylene bag just below the water surface along five equally spaced transects 100 m apart. Samples were concurrently taken at 45- and 90-cm water depths three times a week (three consecutive days) from 11 April to 27 September 2000. Samples were taken once a day from 11 to 27 April between 0730 and 0830 h and twice daily between 0730 and 0830 and 1200 and 1300 h from 2 May to 27 September.

The Chicago Park District (CPD) independently analyzed triplicate water samples from this beach five days a week from 2 June to 1 September; the samples were collected between 0900 and 1000 h.

Diurnal study. To study the effects of insolation and time of day on *E. coli*, 10 days were randomly selected from among predetermined sampling dates between May and September 2000. During each day, water was collected at each of the five transects hourly between 0700 and 1400 h at 45- and 90-cm depths and analyzed for culturable *E. coli* densities.

In situ transparent and dark bag experiment. The aim of this study was to validate the observed relationship between insolation and *E. coli* concentration. On 18 September 2000, 80 sterile, 300-ml polyethylene bags were filled with 175-ml aliquots of lake water collected from a 45-cm depth at 0700 h. Half of the treatment bags were completely covered with duct tape so that no light could penetrate (dark bags), and the remaining half were left transparent. Prior experiments indicated that light absorption through the polyethylene bag was less than 1%. Another 18 bags were filled with sterile buffer water and served as a control. All bags were randomly distributed 20 cm apart on a wire suspended at mid-water at a 45-cm depth. Every hour from 0800 through 1500 h, five each of dark and transparent bags, one control bag, and five ambient water samples were retrieved and analyzed for *E. coli*.

Aside from these bags, 10 blanks were prepared, each with 175 ml of sterile phosphate-buffered water (5 dark, 5 transparent), which were placed out at the beginning of the experiment and retrieved at 1500 h. StowAway TidbiT Temp Logger sensors (Onset, Inc.) were placed in two additional bags (one each transparent and dark). Temperature measurements were logged at 15-min intervals. Both dark and transparent bags tracked ambient water temperature ($\pm 1^\circ\text{C}$).

Hydrometeorology. Wind direction and wind speed, wave height, water temperature, air temperature, cloud cover (identified as cloudy, partly cloudy, hazy, foggy, and sunny), and shoreline distance from a fixed point were recorded in the field throughout the sampling period. In addition, weather conditions were continuously monitored with an onsite Campbell Scientific Instrument weather station and datalogger from 27 May through 30 September 2000. Solar irradiance (insolation) was measured by a Campbell pyrometer and expressed in megajoules per square meter. UV radiation data were obtained from the Environmental Protection Agency UV Monitoring Program (UV-NET) at the nearby University of Chicago. Summed UV data (286.5 to 363 nm) were recorded with a Brewer Spectrophotometer (Kipp & Zonen, Inc., Saskatoon, Canada). Data surrounding each hour were averaged. Photosynthetically active radiation (PAR) and UV radiation were averaged over 5-min intervals 1 m above and 22 cm below the water surface on 18 September 2000 during the microcosm study and on 25 September, using an Apogee Micrologger datalogger. Relative lake level was measured with a Druck pressure transducer, and ambient water conditions (i.e., temperature, specific conductance, turbidity, dissolved oxygen, and redox) were measured every 15 min with a YSI 6600 multiprobe sonde.

Microbiological analyses. All water samples for *E. coli* analysis were processed within 4 h by a membrane filtration method using mTEC agar (2). Generally, three volumes (1, 10, and 50 ml) were filtered for each sample. Yellow colonies on the mTEC plates were confirmed as *E. coli* by the substrate test (urease test); plates with between 20 and 80 colonies were selected for determining *E. coli* counts in the sample, and results were expressed as CFU 100 ml⁻¹.

Statistical analyses. All statistical analyses, except regression trees, were performed with SPSS, version 11.5 (SPSS, Inc., Chicago, Ill.). Regression trees were developed with SYSTAT, version 9 (SPSS, Inc.), using the least-square loss function, 23 splits, 0.05 minimum proportions, a 0.05-split minimum, and a minimum of 5 objects. Regression trees use recursive-partitioning algorithms to develop optimal homogeneous groupings of predictive variables. The dependent variable (*E. coli* in this study) was log transformed to meet parametric assumptions of equality of variances and normal distribution. These assumptions were confirmed by using the Kolmogorov-Smirnov test for normality and Levene's test for equality of variance coupled with examination of cumulative proportions probability (P-P) plots. Hydrometeorological data were not transformed. Significance was set at $P = 0.05$ unless otherwise stated.

RESULTS

***E. coli* and hour of sampling: daily sampling.** Between 2 May and 27 September 2000, *E. coli* counts were higher in the morning than in the afternoon ($P < 0.001$) and morning and afternoon *E. coli* densities were well correlated ($P < 0.001$). Diurnal differences in concentration seemed less pronounced in early spring than later in the summer.

Cloud cover exerted a strong effect on *E. coli* concentrations (Fig. 1). Bacterial counts rarely exceeded swimming criteria (>235 CFU 100 ml⁻¹) on sunny mornings, while on partly cloudy or cloudy mornings, exceedances were common. In the afternoon, variation was somewhat greater, but full sun resulted in relatively low *E. coli* counts: cloudy days often had high *E. coli* counts (above the recommended limit), and partly cloudy days were unpredictable.

Hourly *E. coli* patterns. During the 10 days of hourly sampling, there was a wide range of ambient conditions, and even within each sampling day, conditions often changed between morning and afternoon (Table 1). Generally, wind speed increased while wind direction remained stable.

Five of the 10 sampled days (25 May, 24 July, 7 August, 16 August, and 18 September) had sunny skies. Mean *E. coli* counts for these 5 days fit well an exponential decay curve over time at both 45- and 90-cm depths ($R^2 = 0.98$ and 0.96). The remaining days with variable weather conditions also fit exponential curves over time at both 45- and 90-cm depths ($R^2 = 0.94$ and 0.99) (Fig. 2). For the 5 sunny days, the derived decay function at 45-cm depth was $Y_{45} = 48091e^{-0.4682t}$, where Y is

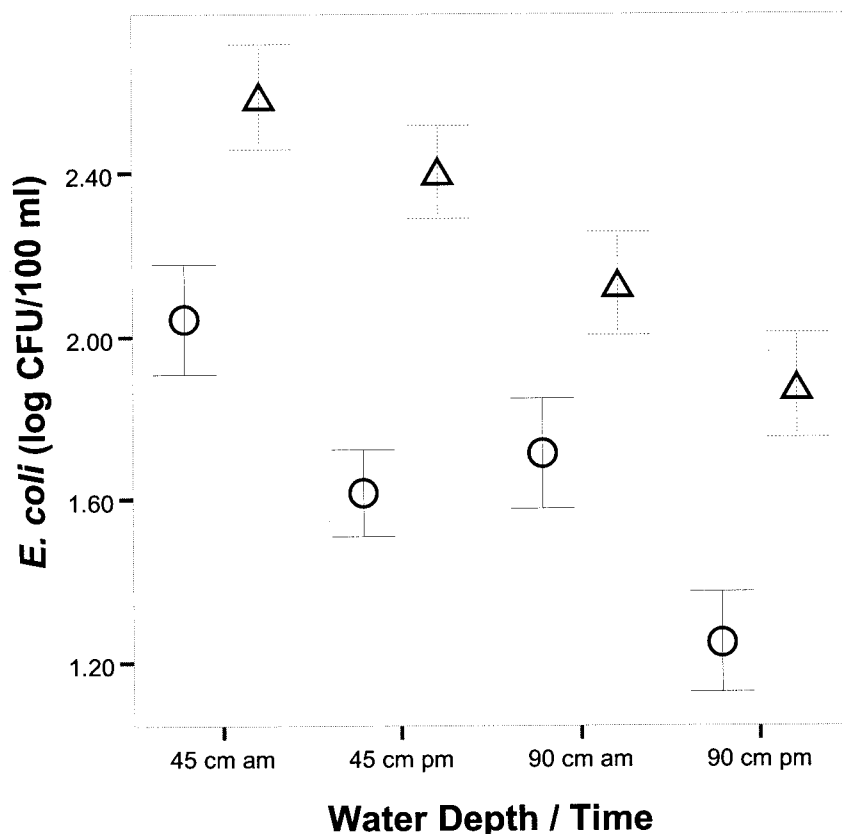


FIG. 1. *E. coli* concentration (mean log transformed) from 4 April to 27 September 2000 during clear and sunny (○) and nonsunny (△; i.e., fog, haze, partly cloudy, or cloudy) mornings and afternoons at 45- and 90-cm water depths. Error bars = ± 1 standard error.

the dependent variable in *E. coli* CFU 100 ml⁻¹ and t is time in hours. The derived decay function for sunny days at a 90-cm depth was $Y_{90} = 12746e^{-0.4184t}$.

For both groups of days, variation between replicates decreased rapidly throughout the day, and by midafternoon, replication error was quite low, especially with the sunny days. Morning mean counts were over 1,000 CFU 100 ml⁻¹ higher than afternoon counts at the 45-cm depth. Surprisingly, rate-

of-change coefficients were higher on the variable days (0.626 day⁻¹ at 45 cm on sunny days, 0.658 day⁻¹ at 90 cm on sunny days, 0.746 day⁻¹ at 45 cm on variable days, and 0.713 day⁻¹ at 90 cm on variable days). *E. coli* counts decreased more rapidly at shallow depths, appearing to converge and perhaps reach equilibrium with *E. coli* counts in 90 cm of water by afternoon (Fig. 2). The sunny days had depressed curves relative to the variable days, but initial counts for the variable days were

TABLE 1. General ambient conditions observed during 10 days randomly selected from among designated sampling days for hourly sampling^a

Date (mo/day/yr)	Cloud cover		Insolation (MJ m ⁻²)	Wind speed (m/s)		Prevailing wind direction (azimuth °)		Temp (°C)		Wave ht (cm)	E. coli count (CFU 100 ml ⁻¹ at depth)			
											45 cm		90 cm	
	a.m.	p.m.		a.m.	p.m.	a.m.	p.m.	Air	Water		Initial	Final	Initial	Final
5/25/00	Sunny	Sunny	0.4	11.3	10.8	NNE	NNE	20.7		13.5	128.4	9.8	2.4	1.0
6/12/00	Cloudy	Partly cloudy						15.4	15.7	29.8	4,160.0	184.0	2,900.0	122.0
6/26/00	Hazy	Partly cloudy		8.3	12.6	SSW	SSW	25.2	18.8	13.5	110.4	14.8	46.8	21.2
7/11/00	Cloudy	Partly cloudy	1.2	8.9	11.9	ENE	NNE	20.7	21.8	14.4	1,500.0	464.0	1,620.0	148.0
7/24/00	Sunny	Sunny	2.1	5.1	10.3	ENE	ENE	20.2	22.8	9.2	89.0	58.5	108.4	34.0
8/01/00	Sunny	Cloudy	1.2	5.3	7.6	WSW	SSW	22.3	23.0	11.1	143.5	131.6	118.0	76.8
8/07/00	Sunny	Sunny	1.8	8.1	11.6	WSW	WSW	25.1	22.9	3.0	6,180.0	79.6	2,984.0	14.0
8/16/00	Sunny	Sunny	1.8	14.4	10.1	NNE	NNE	21.4	23.0	24.1	3,320.0	109.6	1,484.0	79.2
9/18/00	Sunny	Sunny	2.1	8.1	13.0	SSW	SSW	22.2		4.2	236.8	24.0	94.0	12.8
9/25/00	Cloudy	Cloudy	0.4	17.6	17.4	ENE	NNE	12.4		23.7	112.0	88.0	78.0	93.2

^a Field technicians assessed cloud cover at time of sampling. Insolation, air and water temperature, and wave height are averaged from continuously logged data (5-min intervals). Wind speed and wind direction were averaged for morning and afternoon sampling periods. The *E. coli* count is expressed as mean CFU 100 ml⁻¹ for the five transects sampled. The initial and final *E. coli* counts were collected at 0700 h and 1500 h.

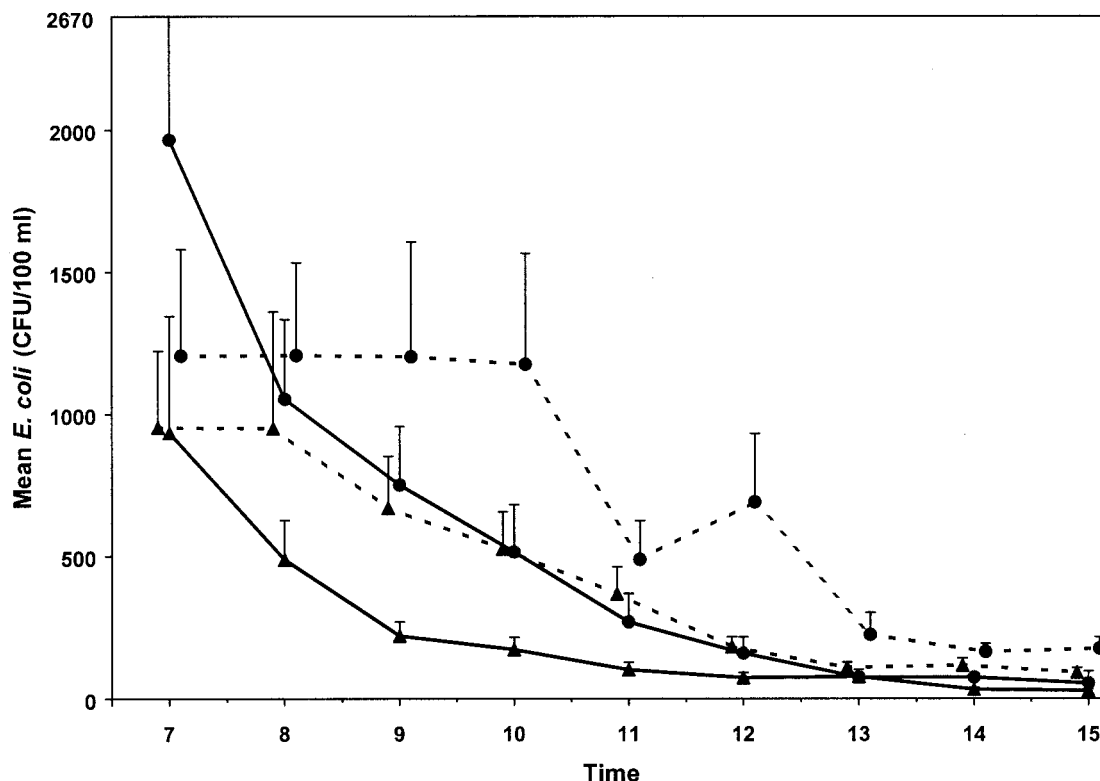


FIG. 2. Mean exponential decay curves for *E. coli* during 10 days randomly selected from among designated sampling days. $n = 5$ for each mean; error bars = +1 standard error. Sunny days (solid lines) and cloudy days (dashed lines) are compared at 45-cm (●) and 90-cm (▲) depths. Decay functions and coefficients of determination are given in the text. Times are in hours.

similar to (45 cm) or below (90 cm) the initial counts of the sunny days.

While the patterns of *E. coli* decline were similar among sampling days, for the 5 days where *E. coli* was significantly correlated with insolation, the magnitude of decline differed (Table 2). The largest decline was on 7 August, with decreases at 45 and 90 cm of 6,100 and 2,970 CFU 100 ml⁻¹, respectively, and only 1.3 and 0.5% remaining. The smallest decrease was on 24 July, with a decrease of only 30 CFU 100 ml⁻¹ and 66% of the initial counts remaining at the 45-cm depth. At 90 cm on that day, the system lost 74 CFU 100 ml⁻¹ over the 8-h period and 31% remained in the system (Table 1).

Certain ambient conditions interfered with the expected patterns of *E. coli* decline relative to insolation, including changes in cloud conditions and wind speed and direction. At both 45- and 90-cm depths on 11 July, there was a significant relationship between insolation and *E. coli* concentration despite cloudy conditions. Cloud cover decreased late in the day, which was sufficient to induce *E. coli* inactivation. On 12 June and 25 September, there was no relationship between *E. coli* counts and insolation, and both days had high waves and onshore winds.

Insolation: light components, and penetration. Atmospheric (1 m above the lake) and submerged (22-cm depth) PAR and UV were continuously measured on 18 (sunny, calm day) and 25 (cloudy, blustery day) September, along with hourly *E. coli* concentrations. On 18 September, a day with gentle south winds, low waves, and so presumably low turbidity, on average, UV was reduced by 48% in the water while PAR decreased by

only 17%. The high penetration of both UV and PAR reaching the water on 18 September may have contributed to the notable decrease in *E. coli* counts throughout the day. On 25 September, a day with a strong east-northeast wind, moderately high waves, and increased turbidity, UV and PAR were effectively reduced (by 94 and 59%, respectively), resulting in minimal effect on *E. coli*.

UV light constitutes a portion of total insolation; on hourly sampling days, UV was 0.11 versus 1.41 MJ m⁻² for total insolation data. There was no clear relationship between UV radiation levels and *E. coli* concentrations ($R = -0.172$, $P = 0.110$) in 45 cm of water, suggesting that *E. coli* inactivation in water was attributable to insolation (total) rather than UV radiation alone. Further, continuous monitoring of light penetration in 45 cm of water on a sunny, calm day versus a cloudy, turbulent day showed that percent absorption of UV light was generally far greater than PAR under both conditions, although this trend was especially notable on a cloudy and turbulent day. On average, only 6% of the ambient UV light remained after passing through 22 cm of lake water on a cloudy day.

Insolation versus hour of sampling. Although there was the expected relationship between time of day and total insolation ($P < 0.001$), exposure time (dosage) was generally a better predictor of *E. coli* density than was insolation (Fig. 3). One reason may be the inherent random factor variation in insolation caused by interactions with cloud cover, smog, fog, season, lake conditions, and turbidity versus the fixed factor of hourly sampling.

TABLE 2. Linear regression of insolation and log *E. coli* concentrations in lake water^a

Result for date (mo/day/yr)	Result at water depth:											
	45 cm						90 cm					
	<i>R</i>	<i>B</i>	SE	β	<i>t</i>	Significance	<i>R</i>	<i>B</i>	SE	β	<i>t</i>	Significance
6/12/00												
Intercept		3.561	0.241		14.777	0.000		3.184	0.271		11.742	0.000
Insolation (MJ/m ²)	-0.648	-1.24	0.552	-0.648	-2.248	0.059	-0.532	-1.032	0.621	-0.532	-1.662	0.140
7/11/00												
Intercept		3.346	0.067		50.089	0.000		3.227	0.056		57.162	0.000
Insolation (MJ/m ²)	-0.945	-0.336	0.044	-0.945	-7.666	0.000	-0.971	-0.395	0.037	-0.971	-10.657	0.000
7/24/00												
Intercept		2.071	0.099		20.96	0.000		2.135	0.094		22.74	0.000
Insolation (MJ/m ²)	-0.674	-0.106	0.044	-0.674	-2.414	0.046	-0.935	-0.289	0.042	-0.935	-6.952	0.000
8/01/00												
Intercept		2.179	0.089		24.454	0.000		2.071	0.085		24.34	0.000
Insolation (MJ/m ²)	-0.434	-0.08	0.063	-0.434	-1.274	0.243	-0.524	-0.097	0.06	-0.524	-1.629	0.147
8/07/00												
Intercept		4.094	0.257		15.92	0.000		3.552	0.254		13.99	0.000
Insolation (MJ/m ²)	-0.897	-0.675	0.126	-0.897	-5.359	0.001	-0.898	-0.673	0.124	-0.898	-5.411	0.001
8/16/00												
Intercept		3.624	0.095		38.088	0.000		3.29	0.119		27.701	0.000
Insolation (MJ/m ²)	-0.976	-0.554	0.046	-0.976	-11.919	0.000	-0.943	-0.436	0.058	-0.943	-7.512	0.000
9/18/00												
Intercept		2.337	0.081		28.791	0.000		2.107	0.075		28.237	0.000
Insolation (MJ/m ²)	-0.918	-0.191	0.031	-0.918	-6.115	0.000	-0.931	-0.194	0.029	-0.931	-6.75	0.000
9/25/00												
Intercept		2.067	0.078		26.378	0.000		1.788	0.095		18.781	0.000
Insolation (MJ/m ²)	-0.292	-0.119	0.148	-0.291	-0.806	0.447	-0.082	-0.039	0.18	-0.082	-0.217	0.835

^a Note that only 8 of the 10 sampled days are represented; pyrenometer readings were unavailable on 25 May and 26 June. Significant relationships are in bold.

For insolation, Pearson's *R* for the 10 hourly sampling days at a 45-cm depth was $-0.65 (\pm 0.10)$ [standard error]. Overall Pearson's *R* ranked slightly higher at a 90-cm depth: $-0.71 (\pm 0.07)$. There was substantial variation among dates sampled. On 5 of the 8 sampling days with insolation data, there was a significant relationship between insolation and *E. coli* counts at 45 cm and also at 90 cm of depth. The highest individual *R* values were at a 45-cm depth ($R = -0.95$ to -0.98). While UV radiation alone (286.5 to 363 nm) was correlated with total insolation ($P < 0.001$), it was a marginally good predictor of *E. coli* concentration at a 90-cm depth ($R = -0.257$, $P = 0.016$) but not at a 45-cm depth ($R = -0.172$, $P = 0.110$).

Solar exposure versus cumulative *E. coli* decrease. Insolation and decrease in culturable *E. coli* for the 5 sunny days of hourly sampling had a variable logarithmic nature (Fig. 3). The steepness of each curve (i.e., inactivation of *E. coli*) was directly related to initial *E. coli* counts in the morning. Asymptotes were usually achieved after exposure to a few megajoules per square meter or by the late morning of a sunny day. When initial *E. coli* densities were low (e.g., on 24 July, 89 CFU 100 ml⁻¹) cumulative dosage had no effect on *E. coli* inactivation ($R^2 = 0.22$).

In general, the magnitude and rate of *E. coli* decline over the day were greater with a higher initial *E. coli* concentration. This is illustrated by the significant correlation between transformed data slope and *y* intercept ($R = 0.816$, $P < 0.001$). *E. coli* counts at 45- and 90-cm depths were strongly correlated

($P < 0.001$), although *E. coli* counts at 45 cm generally remained higher throughout the day. In general, *E. coli* counts at 45 cm were more variable and responsive to changes in insolation. The decreased effect of insolation with increased water depth continued such that *E. coli* in offshore water was not significantly correlated with insolation ($P = 0.319$) (data not presented).

In situ mesocosm experiment. In the mesocosm experiment, initial *E. coli* counts were similar, since all bags were filled from one collection of water taken at 0700 h. While *E. coli* counts in the dark bags remained relatively constant throughout the day, counts in the transparent bags declined rapidly after 1100 h; final *E. coli* concentrations were at least a log unit lower in transparent bags than in dark bags (Fig. 4). Ambient *E. coli* concentrations at 45 cm started out and remained higher than either bag type until 1000 h and then began to decline; at 1500 h, ambient counts were not significantly different from those in transparent bags ($P = 0.05$). Further, *E. coli* counts in transparent bags and ambient water displayed an exponential decline curve similar to that seen in hourly sampling on similar days. On average, the temperature in the transparent bag was only 0.32°C higher than in the dark bag.

Repeated-measures analysis of variation (ANOVA) showed that mean *E. coli* was significantly different with time of day (Tukey's $P < 0.01$). Interaction between time of day and transparent, dark, and ambient treatments was also significant ($P < 0.01$). Pearson correlations showed a significant relationship

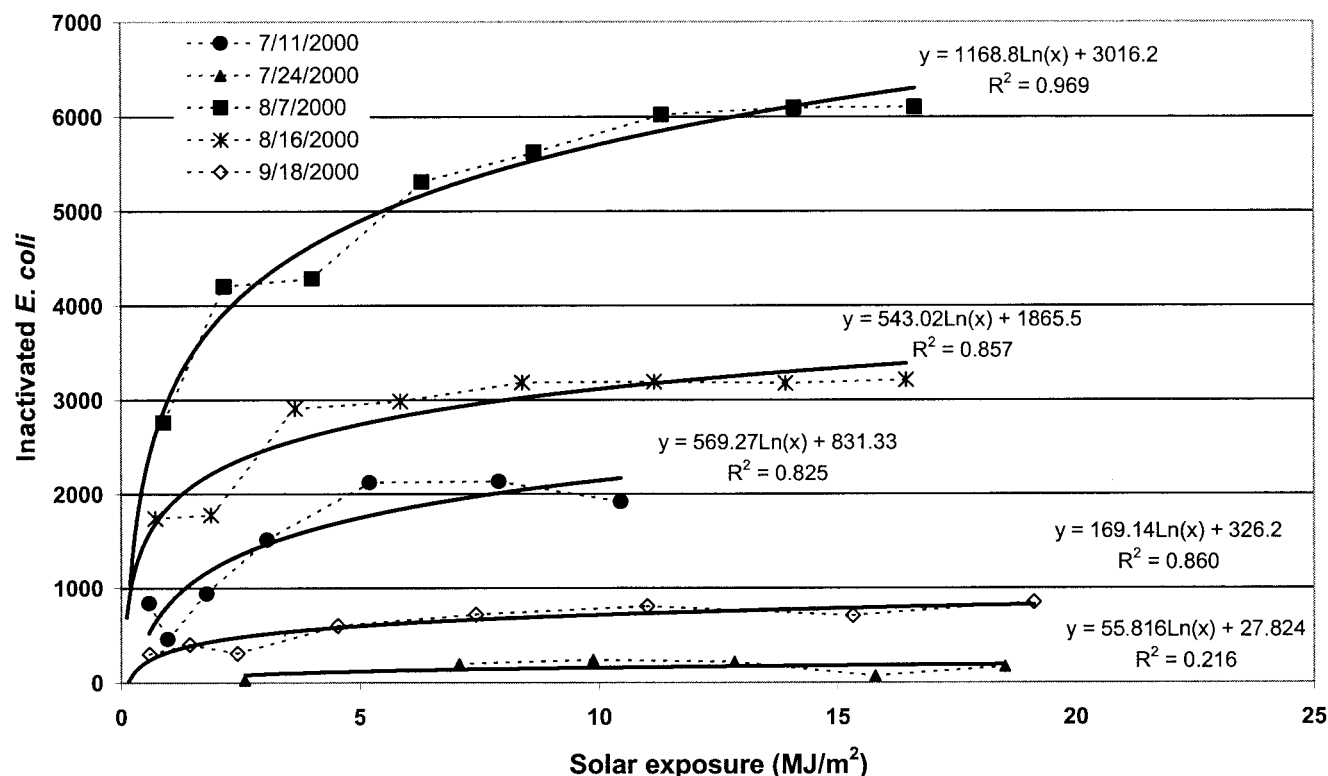


FIG. 3. Actual (dashed lines) and best-fit (solid lines) exponential curves for solar irradiation dosage (expressed as exposure) plotted against total number of inactivated *E. coli*. Linearly expressed best-fit line equations and R^2 are given for each date. Values for 24 July and 18 September have been multiplied by 4 for illustrative purposes.

between *E. coli* counts in transparent bags and ambient water ($R = 0.84$, $P < 0.001$). *E. coli* concentration in dark bags was not correlated with concentration in either transparent bags or ambient water. UV and PAR sensors 1 m above the water surface and at a 22-cm water depth showed an overall increase in irradiance over time until approximately 1300 h; subsequently, light intensity leveled off or decreased. During this day, atmospheric and submerged light readings correlated well ($P < 0.05$), suggesting that atmospheric light readings are a reasonable surrogate for submerged light readings when assessing the effect of insolation on *E. coli*.

Impacts of ambient conditions on *E. coli* counts. Regression tree analysis was used to select predictive variables that best separated *E. coli* data into homogeneous groupings. For 45- and 90-cm depths during both a.m. and p.m., the following model was tested: Mean *E. coli* count = time, insolation, UV radiation, temperature, specific conductance, turbidity, dissolved oxygen, redox, wind speed, barometric pressure, rainfall, relative lake level, wind direction, and wave height.

Only insolation, temperature, and relative lake level had significant homogeneous classification variables ($P < 0.05$) for mean *E. coli* at a 45-cm depth. Of these, insolation was the most important and had an explained variance of 0.404 versus 0.073 for temperature and 0.079 for relative lake level. When insolation was greater than 0.330 MJ m^{-2} , the mean *E. coli* count was $>516 (\pm 796 \text{ [standard deviation]}) \text{ CFU } 100 \text{ ml}^{-1}$. With lower insolation, the mean *E. coli* count was 2,853 ($\pm 2,117$) $\text{CFU } 100 \text{ ml}^{-1}$. Critical temperature and relative lake level cutoffs were 22.5°C and 52 cm, respectively.

DISCUSSION

Distinguishing characteristics of this study. The 63rd St. Beach situation provided an excellent opportunity to study the diurnal patterns of *E. coli* in recreational freshwater for the following reasons. (i) *E. coli* concentrations at this beach are usually high (often over the EPA swimming criterion of 235 $\text{CFU}/100 \text{ ml}$), so changes can be easily observed. (ii) Northern and southern breakwaters isolate the beach from the long current, resulting in semi-embayment of the swimming waters; the resulting beach configuration favors a depositional environment, allowing relatively higher detrital content in foreshore and bottom sediments. (iii) Sunlight penetrates the water column well beyond the swimming area due to the 85:1 slope of the nearshore bottom. Finally, (iv) potential sources of *E. coli* to the water are mostly non-point: chiefly gulls and contaminated foreshore sand (26). Further, the Metropolitan Water Reclamation District of Greater Chicago has built channels and canals to reverse the flow of nearby rivers away from Lake Michigan and divert the contaminated water downstream.

Most studies on solar inactivation of indicator bacteria have been carried out in shallow marine mesocosms (8, 10, 17, 20); no comparable studies for large, freshwater systems, such as the Great Lakes were found in the scientific literature (16). The present study is significant because (i) it is perhaps the first comprehensive research to examine diurnal changes of *E. coli* in a large, non-tidally-influenced temperate freshwater system; (ii) it was a long-duration study—lasting for 6 months; (iii) it

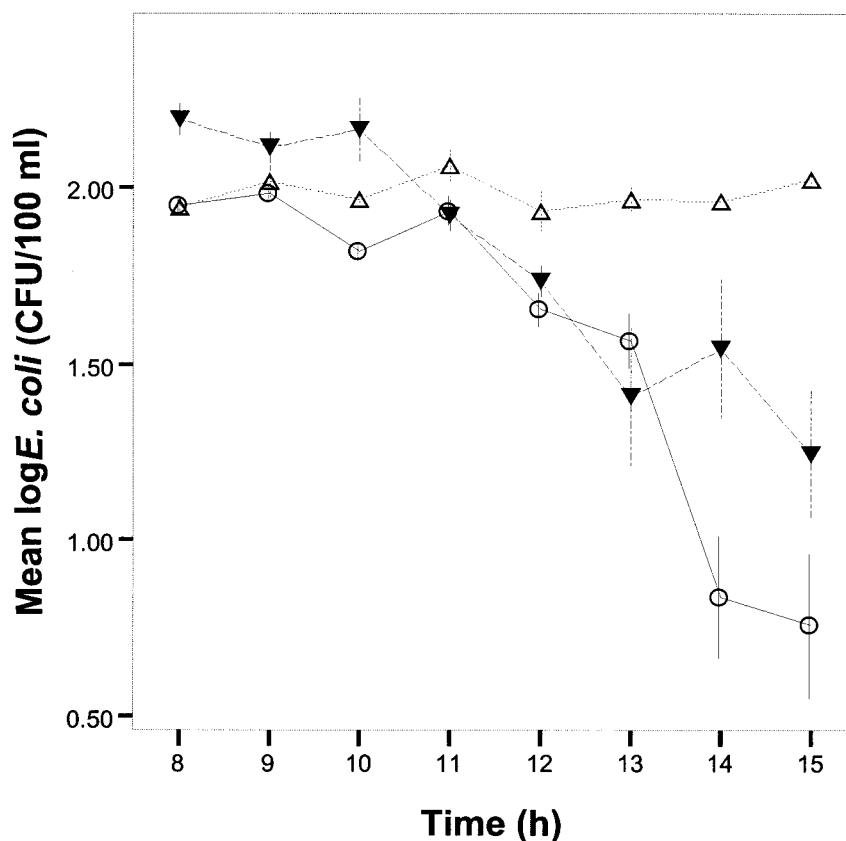


FIG. 4. Effect of insolation on *E. coli* collected at 0700 h and held in in situ mesocosms. *E. coli* counts in dark bags (Δ), transparent bags (○), and ambient lake water (▼) were analyzed each hour on 18 September 2000. Error bars = ± 1 standard error; $n = 5$ for each hour.

included 10 intensive hourly sampling events; and (iv) diurnal changes in *E. coli* densities were measured (a) in situ (at 45- and 90-cm depths where most recreational activities take place and “offshore”), (b) using natural *E. coli* populations, and (c) in the presence of indigenous microbiota.

Influence of time of sample collection. While time of day is not a direct determinate factor, but a correlate for ambient factors such as wind and insolation intensity and dosage, it is a critical element for sampling and also management strategy. When season and atmospheric and lake conditions are normalized, then theoretically time should be a true surrogate for insolation effects. However the microclimate of Chicago’s lake-front coupled with the complexity of shoreline processes complicates predicted relationships substantially. Nonetheless, the effect of time of sampling is probably the most important management and regulatory aspect of diurnal population changes as witnessed in this study. Changing the time of sampling by just a few hours can have a significant effect on *E. coli* results. For example, the CPD monitoring program sampled the same locations at 45 cm as sampled in this study, but typically 2 h later. During this period, CPD data had a beach closure frequency of 43% compared to 64% in this study. Also, water samples collected at 1000 h by CPD had *E. coli* counts 70% lower than in samples collected around 0700 h. From the hourly sampling data, an average decrease in *E. coli* counts between 0830 and 0930 h was calculated as about 220 CFU 100 ml⁻¹ for a clear, sunny day. The decrease between 0830 and

1300 h is far greater: from 1 June to 30 August, daily sampling showed an average decrease of 760 CFU 100 ml⁻¹ for sunny days and 416 CFU 100 ml⁻¹ for cloudy days during these hours. Most encouraging was the effective prediction of day length effect on *E. coli* concentration: using hourly data, the a.m.-to-p.m. difference was calculated within 87%. This predictability, which is driven by the magnitude of insolation, could be useful in developing a management strategy for swimming later in the day without resampling, but it does not help to explain differences between days.

Although insolation was the most influential factor affecting *E. coli* counts in this study, as determined by stepwise regression ($P < 0.001$), a myriad of interacting factors have lesser or secondary effects on *E. coli* concentrations. The results of regression trees show that while there were many interacting factors affecting light penetration (cloud cover, turbidity, wave conditions, relative lake level, time of day) and several that were independent of insolation (water temperature, antecedent rainfall, and water quality parameters), the number of useful parameters can be distilled to only a few: in this case, insolation followed by temperature and relative lake level. Alternately, wave height worked well when relative lake level was omitted. UV radiation was not a useful surrogate for total insolation. For managers, the specific factors that influence the optical properties of water may be less interesting than the consequences of light as a limiting factor in the occurrence of *E. coli*. Nonetheless, it is these factors, along with other ambi-

ent conditions, that act collectively to determine the *E. coli* levels of particular swimming water. Understanding these processes helps to partition ambient from anthropogenic effects, indicator bacterial population dynamics from pathogen response, and health implications from artifacts due to indicator species idiosyncrasies.

Inactivation mechanisms. The process of inactivation (by sunlight) of fecal indicator bacteria in natural waters is rather complex; however, the two major pathways involved in this process appear to be photobiological (DNA damage) and photooxidation (oxidation of cellular components). Studies have shown that oxygen and organic matter (lignins and humic or fulvic acid) can hasten the process of photooxidation damage (18, 19, 25) by producing destructive agents, such as oxygen free radicals (O_2^-) and hydrogen peroxides. In large lakes such as Lake Michigan, photochemical damage can presumably occur besides photooxidation, because the required ingredients—oxygen and organic matter—are available through constant circulation/wave action and surface runoff (or other sources).

While UV radiation causes significant damage (photobiological) to *E. coli*, the amount of these shorter wavelengths reaching earth's surface is approximately only 5% of the total available insolation. Further, UV radiation is easily scattered by particulate material suspended in the atmosphere and lake water. While UV and total insolation were well correlated ($P < 0.001$), the relationship between the bandwidths was likely influenced by angle of incidence, water color, particulates, surface conditions, angle of incidence, and depth. The data suggest that UV radiation has only a minor effect on *E. coli*, relative to total insolation, in all but the upper surface of water or in shallow or clear waters.

The stereotypic exponential decay curve of *E. coli* with insolation was influenced by variation in lake and sky conditions. The 7th of August was a typical summer day with clear skies and offshore winds (i.e., calm lake surface). The exponential decay curve and the response to insolation are evident and similar to the mesocosm experiment results described earlier on 18 September (Fig. 4). In contrast, 25 September was the most overcast day, with blustery onshore winds. *E. coli* remained in relative equilibrium throughout the day, suggesting that (i) when insolation is diminished (possibly in combination with high turbulence), *E. coli* concentration remains unchanged from early morning (and presumably nighttime) levels, and (ii) in a well-mixed system and with decreased insolation, import and export remain relatively equal. The influence of light is further evidenced by the decreased periodicity with increased water depth. While the influence of light on *E. coli* inactivation was apparent at both depths (45 and 90 cm), others have noted inactivation only at shallower depths (Rhodes and Kator, Abstr. Annu. Meet. Am. Soc. Microbiol., 1984), suggesting a relationship between water clarity and depth of effective light penetration. Initial levels of *E. coli* greatly influenced the slope's magnitude ($P < 0.001$). For instance, *E. coli* counts on 12 June dropped nearly 1 log over the day even though insolation was relatively low until late in the day.

There were several instances of a rapid *E. coli* response to diurnal changes in insolation; however, limited replication and high variation made trend confirmation difficult. The pattern on 1 August illustrates a more complex insolation-*E. coli* in-

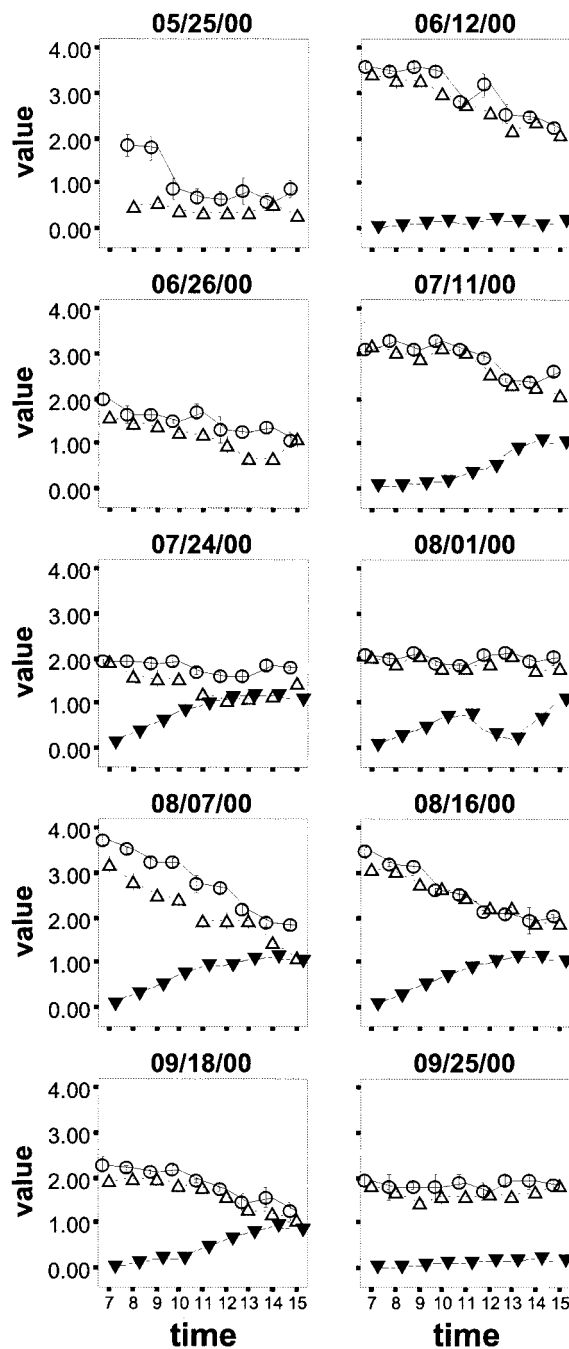


FIG. 5. Relationship between *E. coli* concentration and time of day for 10 days (shown as month/day/year) randomly selected from among designated sampling days. The *E. coli* concentration at 45 (○) and 90 (△) cm is given in log CFU 100 ml⁻¹, and insolation (▼) is given in megajoules per square meter. Error bars = ± 1 standard error; $n = 5$ for each *E. coli* mean. Hourly insolation data were averaged over 5-min intervals.

teraction (Fig. 5). Close inspection shows that *E. coli* declined during sunny conditions in the morning and late afternoon, but this trend was reversed when insolation diminished with cloud cover midday. The rapidity of the response reflects not only the potency of insolation but also steady importation of *E. coli*, consistent with nighttime replenishment observations.

Nighttime *E. coli* replenishment. One of the most intriguing and perhaps revealing questions arising from the study is the unresolved mechanism of nighttime replenishment of *E. coli*. The source may be intrinsic (recovery of nonculturable cells) or extrinsic (importation of additional *E. coli*). The most plausible candidates for external loadings of *E. coli* include drifts from offshore and washing of *E. coli*-rich sand along the swash zone (1, 26). Growth or resuscitation remains a possibility during warmer periods, although the quick rebound in population overnight seems unlikely. Macrophytic algae has been suggested as a potential source of organic material that could account for some population spikes in *E. coli* (7, 27), but in this study, diurnal effects and slopes were similar at a wide variety of temperatures and seasons. In the in situ microcosm experiment, the lack of *E. coli* increase in dark bags suggests that there was no short-term potential for *E. coli* resuscitation. While there was no statistical difference between *E. coli* numbers in ambient water and transparent bags, a side experiment examining bag effects showed there was a tendency for *E. coli* in the bags to be lower in concentration. This difference may be the effect of the bag (e.g., lack of circulation or exposure) or may be due to the introduction of bacteria from offshore or nearshore sources. Attempts to resuscitate lake *E. coli* exposed to full atmospheric sunlight for as little as 30 min have consistently failed even under ideal laboratory conditions (data not shown). The rate of replenishment might be derived from the slope of population recovery with a sudden increase in cloudiness, but this would not take into account the sustained effect of residual insolation. Ideally, sampling over the entire 24-h period would help resolve these questions.

Conclusions. In conclusion, the present findings are consistent with previous mesocosm studies, suggesting that sunlight is among the most potent abiotic factors in the inactivation or killing of indicator bacteria in environmental waters. While it is clear that at 63rd St. Beach insolation has a major influence on *E. coli* concentration, its relative effect likely varies at other beaches and is influenced by circulation, exposure, turbidity, bottom slope, and background levels of *E. coli*.

The response of *E. coli* is predictable and is generally related to the sky and lake conditions. Three lines of evidence support this assessment: (i) *E. coli* concentrations in water declined exponentially as a function of time of day; based on our regression models, a mean decrease in ambient *E. coli* of over 900 CFU 100 ml⁻¹ could be achieved in the first sampling hour; (ii) insolation, UV, and PAR readings reflected corresponding *E. coli* densities in the water; and (iii) controlled, in situ incubation of water in transparent and dark bags suggests that the decrease in *E. coli* densities in water was due to sunlight alone.

The vulnerability of *E. coli* to changing ambient conditions complicates its use as a routine indicator of relative sewage input. In this study, peak levels of *E. coli* were more a function of insolation and lake dynamics than pollution events; however, similar processes would be anticipated regardless of indicator bacterial sources. The study adds to growing evidence of the periodicity of indicator bacterial concentrations in marine and freshwater, which is driven, in large part, by recurring hydrometeorological events such as insolation and tides. The general presumption of the static nature of *E. coli* in natural freshwater has given way to a more dynamic and predictable

model, and this predictability may allow us to understand the nature, source, and fate of this fecal indicator organism in natural waters. That information, in turn, will help in the development and application of more effective approaches for overseeing recreational water quality.

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